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Photosynthetic Pigments of oceanic Chlorophyta belonging to prasinophytes clade VII.

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Abstract

The ecological importance and diversity of pico/nano-planktonic algae remains poorly studied in marine waters, in part because many are tiny and without distinctive morphological features. Amongst green algae, Mamiellophyceae such as *Micromonas* or *Bathycoccus* are dominant in coastal waters while prasinophytes clade VII, yet not formerly described, appear to be major players in open oceanic waters. The pigment composition of 13 strains representative of different sub-clades of clade VII was analyzed using a method that improves the separation of loroxanthin and neoxanthin. All the prasinophytes clade VII analyzed here showed a pigment composition similar to that previously reported for RCC287 corresponding to pigment group prasino-2A. However we detected in addition astaxanthin for which it is the first report in prasinophytes. Among the strains analyzed the pigment signature are qualitatively similar within sub-clades A and B. In contrast RCC3402 from sub-clade C (*Picocystis*) lacks loroxanthin, astaxanthin and antheraxanthin. For sub-clades A and B, loroxanthin was lowest at highest light irradiance suggesting a light–harvesting role of this pigment in clade VII as in *Tetraselmis*.

Keywords : phytoplankton, picoplankton, prasinophytes, pigments, HPLC

The paraphyletic group of prasinophytes is an assemblage of free-living unicellular microalgae present in both marine and freshwater habitats (Leliaert et al. 2012). Molecular phylogenetic, ultra-structural, and biochemical approaches have helped taxonomists to re-organize gradually the group into new classes and clades (Guillou et al. 2004, Marin and Melkonian 2010, Subirana et al. 2013, Lemieux et al. 2014a). Currently the prasinophytes are divided into nine groups known as clades I to IX, based on phylogenetic analyses of the nuclear 18S (nuclear-encoded small subunit rRNA) gene (Fawley et al. 2000, Guillou et al. 2004, Viprey et al. 2008). These clades may correspond to true classes, or be composed of a small number of species or of environmental sequences only. For example, Chlorodendrophyceae (Massjuk 2006) known previously as prasinophytes clade IV was recently raised to the class level and added to the "core of chlorophytes" (Fucikova et al. 2014). Clade V corresponds to the order Pycnococaceae with two major species, *Pseudoscourfieldia marina* and *Pycnococcus provasolii* which are probably two forms of a single life cycle (Fawley et al. 1999, Guillou et al. 2004). Clades VIII and IX are composed entirely by environmental sequences without representatives in culture (Viprey et al. 2008). Clade II, previously corresponding to the order Mamiellales, was raised recently to the class level as Mamiellophyceae (Marin and Melkonian 2010) and contains three important genera of marine pico-phytoplankton: *Micromonas* (Butcher 1952), *Bathycoccus* (Eikrem and Throndsen 1990) and *Ostreococcus* (Chrétiennot-Dinet et al. 1995).

In coastal waters, Mamiellophyceae appear largely dominant, especially within the pico-plankton, with the genus *Micromonas* making the highest contribution and followed to a lesser extent by *Bathycoccus* (Throndsen, J. and Kristiansen 1991, Not et al. 2004, Collado-Fabri et al. 2011, Balzano et al. 2012). In contrast in the open ocean, another group of prasinophytes, clade VII, with cell size in the 3 to 5 µm range, has been found to make an important contribution to

the pico-plankton community in regions such as the Equatorial Pacific and Mediterranean Sea (Moon-van der Staay et al. 2000, Viprey et al. 2008, Shi et al. 2009). The distribution of clade VII in typically oceanic mesotrophic waters makes this an interesting group. Prasinophyte clade VII contains several cultured strains, but it has not been described formerly yet. Guillou *et al.* (2004) divided this group into three well-supported sub-clades, A, B and C, the latter being formed by *Picocystis salinarum*, a small species found in saline lakes (Lewin et al. 2000, Roesler et al. 2002, Krienitz et al. 2012).

Traditionally, pigment signature has been used to determine the taxonomy of algae groups present in the water column (Jeffrey et al. 1997). This approach has been largely superseded by molecular approaches (Liu et al. 2009) but pigments remain an important phenotypic characteristic that allowed to point out the importance of green algae in specific regions of Pacific Ocean, Mediterranean Sea or Arctic Ocean (Obayashi and Tanoue 2002, Miki et al. 2008, Gutiérrez-Rodríguez et al. 2010, Coupel et al. 2014). The use of pigments in order to discriminate different types of prasinophytes has proven to be a difficult task since a diversity of photosynthetic signatures can be found in this group. Prasinophytes can be divided into three major groups based on their carotenoid composition (Egeland et al. 1997, Garrido et al. 2009). Group 1 contains the basic set of carotenoids present in Chlorophyceae: neoxanthin, violaxanthin, lutein, zeaxanthin, antheraxanthin, astaxanthin and β-β-carotene. The two later pigments are found to accumulate in *Haematococcus* and *Dunaliella* species under stress conditions (Lemoine and Schoefs 2010). Group 2 consists of the basic set of carotenoids plus loroxanthin (2A) and siphonaxanthin (2B). Group 3 contains prasinoxanthin (3A) and uriolide/micromonal (3B) in addition to the pigments found in group 2 (Jeffrey et al. 2011)

Within clade VII, a single strain RCC287 has been analyzed for its pigment composition (Latasa et al. 2004). A large number of clade VII strains are available from the Roscoff Culture Collection (http://roscoff-culture-collection.org/) originating from a range of environment. Their genetic diversity is currently analyzed allowing further delineation of new sub-clades (Lopes et al. in preparation) within the two major sub-clades A and B described by Guillou *et al* (2004). Among the 44 strains of clade VII present in the RCC, 39 belong to sub – clade A. The aim of this study was to determine the phenotypic characteristics of this important group of marine green algae by analyzing the pigment composition of thirteen strains belonging to the three sub-clades (A, B, C) of prasinophytes clade VII and isolated from a range of oceanic location and depths (Table 1). We also assessed the effect of three light irradiances on pigment composition for a subset of these strains.

Thirteen strains belonging to clade VII (Table 1) were grown at 22°C in K seawater medium (Keller et al. 1987) under 140 $\mu E.m^{-2}.s^{-1}$in continuous light. A subset of nine strains was also grown at two other light levels (14 and 65 $\mu E.m^{-2}.s^{-1}$). Prior to sample collection, cell concentration was determined by flow cytometry using a Becton Dickinson Accuri C6. Approximatively 50 ml of cultures were harvested in late exponential or early stationary phase and were filtered onto glass fiber GF/F (Whatman, Maidstone, UK) without vacuum and immediately frozen in liquid nitrogen and stored at -80°C. Pigments were analyzed within one month. Frozen filters were extracted with 3 mL of 90% acetone in screw cap glass tubes with polytetra-fluoroethylene (PTFE) lined caps, placed in an ice-water bath. After 15 minutes, filters were homogenized using a stainless steel spatula for filter grinding. Tubes were placed in an ultrasonic bath with water and ice for 5 minutes. The slurries were then centrifuged 5 minutes at 4.500 r.p.m. and supernatants filtered through 13 mm diameter polypropylene syringe filters (MS

PTFE, 0.22 μm pore size) to remove cell and filter debris. Before injection 1ml of each sample extract was added with 0.4 ml of Milli-Q water to avoid peak distortion. Pigments extracted from clade VII strains were analyzed using a modification of Zapata *et al*. (2000) method, described by Garrido *et al*. (2009) to improve the separation of loroxanthin and neoxanthin (Table S1). All graphs and analyses were performed with the R software using the ggplot2 and FactoMineR libraries (R Development Core Team 2013).

Intracellular chlorophyll (Chl) *a* content ranged from 4 to 26 fg per cell in most strains except for RCC996 (VIIA) and RCC3402 (*Picocystis* - clade VIIC) for which it was much higher (Table 2). This range agreed with values previously determined for marine microalgae in the same size range (Simon et al. 1994).

All the prasinophytes clade VIIA and B analyzed here showed a very similar pigment composition (Table 2). It did not seem to change drastically with between sub-clades A and B, nor with the depth of isolation (Figure 1). This composition is very similar to that reported for RCC287 by Latasa *et al.* (2004) corresponding to pigment group prasino-2A. We did not observe strong differences (Figure 1, Table 2) with the data of Latasa *et al.* (2004): in particular the ratios obtained for zeaxanthin and lutein, were very similar in both studies despite the slight difference in light levels (100 *vs.* 140μE.m^{-2}.s^{-1} in our study): zeaxanthin, 0.042 (w/w) *versus* 0.043 (w/w) and lutein, 0.382 (w/w) *versus* 0.363 (w/w). However their study used a less resolutive method and did not report the presence of loroxanthin and astaxanthin in RCC287. For loroxanthin this is probably due to the co-elution of this pigment with neoxanthin in the analytic method employed by these authors. For astaxanthin, our strains were grown in continuous light in contrast to Latasa *et al.* (2004) who used L:D cycles. This might have triggered the synthesis and accumulation of

this carotenoid, which is known to be synthesized under stressful conditions, such as high light, UV irradiation or nutrient depletion in Chlorophyceae, like *Haematococcus* and *Dunaliella* (Lemoine and Schoefs 2010) as well in some strains of Trebouxiophyceae such as *Picochlorum* (Lubián et al. 2000). In *Haematococcus*, maximal production of astaxanthin has been obtained under to continuous illumination (Domínguez-Bocanegra et al. 2004)

In our study, only RCC1124 (sub-clade A) did not contain loroxanthin within strains belonging sub-clades A and B (Table 2). *Picocystis* (RCC3402, clade VIIC) did not contain loroxanthin, astaxanthin and antheraxanthin (Table 1). Violaxanthin and lutein were the most abundant carotenoids for clade VIIA and VIIB. Astaxanthin came as the third more abundant within VIIA and RCC2337 (VIIB), while β-ε-carotene came third for RCC2339 (VIIB). For *Picocystis* (clade VIIC), lutein and β-ε-carotene were the most abundant carotenoids (Figure 1, Table 2), but the ratio of accessory pigments to Chl *a* was much lower than in clades VIIA and B (Figure 1).

We analyzed the influence of irradiance (14, 65 and 140 $\mu E.m^{-2}.s^{-1}$) on pigment composition of nine strains of prasinophytes VIIA and B (Figure 2 and S1, and Table S2). Change in light intensities induce opposite trends in the ratios of photosynthetic and photoprotective pigments relative to Chl *a*. Accessory chlorophylls and carotenoids involved in light capture either increase relative to Chl *a* at low light, while photoprotective carotenoids increase at high light (Schlüter et al. 2000, Henriksen et al. 2002). In our study, Chl *b* ratios increased slightly at low light, as expected, except for RCC3376 that showed a very slightly lower ratio at low light than at high light (0.78 *vs.* 0.81) (Figure 2 and Table S2). A similar slight decrease was also observed by Garrido *et al*. (2009) for the green alga *Tetraselmis suecia*.

Neoxanthin, β-β carotene and loroxanthin appeared to have a light harvesting role for most of the strains, except RCC287 (Figure 2 and Table S2), but change could be subtle as in the case of neoxanthin. Neoxanthin has been found to be associated with light harvesting complexes in the Mamiellophyceae *Mantoniella squamata* (Wilhelm and Lenarz-Weiler 1987). The most drastic changes were observed for loroxanthin (Figure 2) suggesting that this pigment has a major light harvesting role in clades VII A and B in agreement with what observed by Garrido *et al*. (2009) with *T. suecica*. One strain, RCC3376 (sub-clade A), had very low ratios of loroxanthin to Chl *a* which did not seem to change with light (Figure 2).

The increase of astaxanthin (from 2 to 4-fold depending on the strains) with light intensity suggests that this carotenoid has a photoprotective role for in clade VIIA and B (Figure 2), as previously demonstrated in the Chlorophyceae *Haematococcus pluvialis* (Wang et al. 2003, Gao et al. 2012). Among all strains, RCC3374 showed the most impressive accumulation of astaxanthin which contributed up to 42% of the total carotenoid pool under high light conditions (Figure 2). In comparison, *H. pluvialis* can accumulate 86 - 90% of astaxanthin in the total carotenoid pool after sixteen days cultures of under stress conditions (Sarada et al. 2002). However RCC3374 cell size (3 µm) is about 7 times smaller than *H. pluvialis* resting cells (25 µm, Gu et al. 2013) and maximum cell density is 3.10^6 cell mL^{-1}. A rough computation show that our RCC3374 culture was able to produce only 0.006 mg/L of astaxanthin against 8.3–10.69 mg/l found in old cultures of *H. pluvialis* (Sarada et al. 2002). If one could increase the cell concentration about 1000-fold with a different medium and manipulate astaxanthin cell content through stress, then clade VII could be an interesting contender for astaxanthin production.

Lutein also showed a photoprotective behavior. Its contribution to total carotenoids increased sharply from low to medium light and the stabilized at the highest light (Figure 2, Table S2). Such increase under high light conditions has been previously reported by Böhme et al. (2002) in the Mamiellophyceae *M. squamata*. These authors suggested that lutein played an important role as intermediate of biosynthesis for light harvesting pigments after light shifts from HL to LL. This role was coherent with its loose binding to the LHC apoprotein, also observed for the violaxanthin cycle (VAZ) carotenoids. However, lutein and loroxanthin are xanthophylls derived from β-ε carotene, and both have also been suggested also to take part in photoprotective mechanisms (non-photochemical quenching, NPQ) to prevent photo-oxidative damage in high light conditions in the green alga *Chlamydomonas reinhardtii* (Niyogi et al. 1997).

As for lutein, the content of the photoprotective xanthophyll cycle involving violaxanthin, antheraxanthin and zeaxanthin (VAZ cycle) relative to Chl *a* increased in general sharply from low to medium light and the stabilized at high light (Figure 2 and S1, Table S2). Their specific behavior however differed among strains. For example, zeaxanthin did not change much in RCC287 and RCC857 while it increased several-fold in other strains (e.g. RCC719, Figure 2).

In order to assess the visualize any hidden relationship between strain origin and pigment composition, we performed a Principal Component Analysis (PCA) based on pigments to Chl *a* ratios (Figure 3). The first two components explained more than 50% (dimension 1 and 2, 33.1 % and 20.4 %, respectively) of the variance. Pigments contributing positively to dimension 1 corresponded to photoprotective pigments (lutein, β-ε carotene, zeaxanthin, violaxanthin, antheraxanthin and astaxanthin) while pigments involved in photosynthetic capture, such as loroxanthin and β-β–carotene, contributed negatively to this axis. Pigments with moderate

response to light, such as Chl *b* and neoxanthin, contributed to dimension 2. Strains under medium and high light were separated from those at low light along dimension 1 which reflects that for several pigments the largest changes occurred between low and medium light and then stabilized at high light. This analysis confirmed that pigment composition and change did not seem to be linked to strain sub-clade (A vs. B) or depth of isolation (surface *vs*. DCM).

Recent phylogenetic results pointed clade VII A/B as a sister group of the core of Chlorophyta (Guillou et al. 2004, Leliaert et al. 2012, Lemieux et al. 2014a, 2014b). The presence of astaxanthin as in core Chlorophyta while it is absent with other prasinophytes may reflects another common feature between clade VII and core Chlorophyta. Moreover while Guillou *et al* (2004) included *Picocystis* into clade VII based on the phylogenetic analysis of 18S rRNA gene, the recent analysis of chloroplast genomes (Lemieux et al. 2014a) has shown widely divergent traits between *Picocystis* on one side and sub-clades VIIA and B, on the other side. The divergent carotenoid composition of *Picocystis* (absence of loroxanthin, astaxanthin, and antheraxanthin) reinforce these phylogenetic analyses.

Some of the important findings of this study include the discovery of loroxanthin and astaxanthin in prasinophytes clades VIIA and B, which had not been reported before, as well the possible role of the former pigment as a light harvesting accessory pigment. This should prompt the need to reexamine the pigment composition of other members of this diverse and ancient group constituted by prasinophytes using improved analytical protocols.

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Table 2. Concentration of Chl *a* per cell, ratios of pigment to Chl *a* concentration and contribution to total carotenoids (in italics) for thirteen strains of prasinophytes clade VII (Chlorophyta) grown at 140 $\mu E.m^{-2}.s^{-1}$.

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Figure 2. Change in pigment to Chl *a* ratios for Chl *b* and five major carotenoids in nine strains of prasinophytes clade VII under three light intensities. Top: pigments involved in light capture. Bottom: pigments involved in photoprotection. Solid lines correspond to sub-clade VIIA and dashed lines to VIIB. Open symbols correspond to surface strains, closed ones to DCM strains and grey to unknown depth of isolation.

Figure 3. Principal component analysis using the pigment to Chl *a* ratios as variables for the strains grown at 3 light levels (Table S2). Top. Variables. Bottom. Samples. Circles correspond to clade VIIA and triangles to clade VIIB. Closed symbols correspond to low light, grey symbols to medium light and open symbols to high light.

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Figure S1. Change in pigment to Chl *a* ratios for Chl *b* and four minor carotenoids in nine strains of prasinophytes clade VII under three light intensities. Solid lines correspond to clade VIIA and dashed lines to VIIB. Open symbols correspond to surface strains, closed ones to DCM strains and grey to unknown depth of isolation.

Table 1

RCC	Sub-clade	Strain name	Other names	Ocean origin	Region origin	Depth Isolation (m)
15	A	CCMP 1205		NA		NA
287	A	NOUM15	NOUM97015	Pacific Ocean	Equatorial Pacific	120
719	A	IndianOcean_45-8		Indian Ocean		76
856	A	Biosope_42 A2	CCMP3325	Pacific Ocean	Marquesas islands	10
857	A	Biosope_40 A2		Pacific Ocean	Marquesas islands	10
996	A	Biosope_46 B4S		Pacific Ocean	South East Pacific	100
998	A	Biosope_46 C3S	NIES2676, CCMP3334	Pacific Ocean	South East Pacific	100
1124	A	PAP_AD	PAP_Ludwig_AI	Atlantic Ocean	North Atlantic, PAP site	10
3374	A	CCMP 2152	A7831	Pacific Ocean	Hawaii	NA
3376	A	CCMP 2113	A9533	Pacific Ocean		85
2337	B	JST MH335	MH335, NIES2756	Pacific Ocean	Iki Island	0
2339	B	JST MH340	MH340, NIES2758, CCMP3360	Pacific Ocean	Iki Island	0
3402	C	CCMP 1897	SFBB	Pacific Ocean	San Francisco Bay	0

Remark : Ordered by clade and then RCC number

Table 2

Strain	sub - clade	Light	fg Chl *a*	Chl *b*	Chlide *a*	Chlide *b*	Sum of carotenoids	Carotenoids															
								Loroxanthin		Neoxanthin		Violaxanthin		Astaxanthin		Antheraxanthin		Zeaxanthin		Lutein *a*		ββ - carotene		βε - carotene	
		µE.m^{-2}.s^{-1}	/cell	/Chl *a*	/Chl *a*	/Chl *a*	/Chl *a*	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%
RCC15	A	140	20.3	0.90	0.066	0.018	1.32	0.058	*4.4*	0.003	*0.2*	0.327	*24.8*	0.165	*12.5*	0.043	*3.3*	0.103	*7.8*	0.376	*28.4*	0.154	*11.7*	0.093	*7.0*
RCC287	A	140	5.0	0.99	0.106	0	1.53	0.024	*1.6*	0.167	*10.9*	0.572	*37.3*	0.210	*13.7*	0.024	*1.6*	0.043	*2.8*	0.363	*23.7*	0.051	*3.3*	0.079	*5.2*
RCC719	A	140	14.9	0.68	0.000	0	1.93	0.035	*1.8*	0.086	*4.4*	0.617	*32.0*	0.264	*13.7*	0.000	*0.0*	0.478	*24.8*	0.193	*10.0*	0.107	*5.5*	0.149	*7.7*
RCC856	A	140	23.4	0.86	0.000	0	2.08	0.113	*5.4*	0.079	*3.8*	0.291	*14.0*	0.570	*27.4*	0.042	*2.0*	0.310	*14.9*	0.461	*22.2*	0.075	*3.6*	0.136	*6.5*
RCC857	A	140	4.1	1.00	0.000	0	1.40	0.026	*1.9*	0.122	*8.7*	0.534	*38.2*	0.187	*13.4*	0.024	*1.7*	0.094	*6.7*	0.291	*20.8*	0.056	*4.0*	0.064	*4.5*
RCC996	A	140	51.6	0.93	0.074	0	1.26	0.043	*3.4*	0.095	*7.5*	0.119	*9.5*	0.191	*15.2*	0.039	*3.1*	0.245	*19.5*	0.364	*28.9*	0.091	*7.2*	0.074	*5.9*
RCC998	A	140	26.1	0.78	0.000	0	1.90	0.014	*0.8*	0.117	*6.1*	0.877	*46.1*	0.157	*8.2*	0.058	*3.0*	0.168	*8.8*	0.285	*15.0*	0.095	*5.0*	0.131	*6.9*
RCC1124	A	140	8.6	0.96	0.099	0	1.47	**0.000**	*0.0*	0.116	*7.9*	0.525	*35.7*	0.148	*10.1*	0.020	*1.4*	0.086	*5.8*	0.399	*27.1*	0.095	*6.5*	0.082	*5.6*
RCC3374	A	140	4.1	0.73	0.229	0	1.84	0.012	*0.7*	0.082	*4.5*	0.272	*14.8*	0.778	*42.3*	0.041	*2.3*	0.118	*6.4*	0.332	*18.1*	0.059	*3.2*	0.146	*7.9*
RCC3376	A	140	4.1	0.81	0.105	0	1.53	0.008	*0.5*	0.112	*7.3*	0.572	*37.5*	0.324	*21.3*	0.054	*3.6*	0.129	*8.5*	0.048	*3.1*	0.085	*5.6*	0.194	*12.7*
RCC2337	B	140	4.4	0.88	0.394	0.236	2.19	0.031	*1.4*	0.137	*6.3*	0.504	*23.1*	0.302	*13.8*	0.051	*2.3*	0.154	*7.0*	0.706	*32.3*	0.061	*2.8*	0.240	*11.0*
RCC2339	B	140	14.6	0.62	0.000	0	1.52	0.026	*1.7*	0.078	*5.1*	0.613	*40.4*	0.045	*3.0*	0.024	*1.6*	0.074	*4.8*	0.302	*19.9*	0.028	*1.8*	0.330	*21.7*
RCC3402	C	140	131.1	0.26	0.016	0	0.54	**0.000**	*0.0*	0.048	*8.9*	0.014	*2.7*	0.000	*0.0*	0.000	*0.0*	0.071	*13.1*	0.200	*37.0*	0.016	*3.0*	0.191	*35.4*
RCC287 [a]	A	*100*	*Nd*	*1.31*	*Nd*	*Nl*	0.76	*Nd*	*Nd*	*0.074*	*9.7*	*0.131*	*17.3*	*Nd*	*Nd*	*0.051*	*6.7*	*0.042*	*5.5*	*0.382*	*50.3*	*0.026*	*3.4*	*0.053*	*7.0*

nd: Not determined

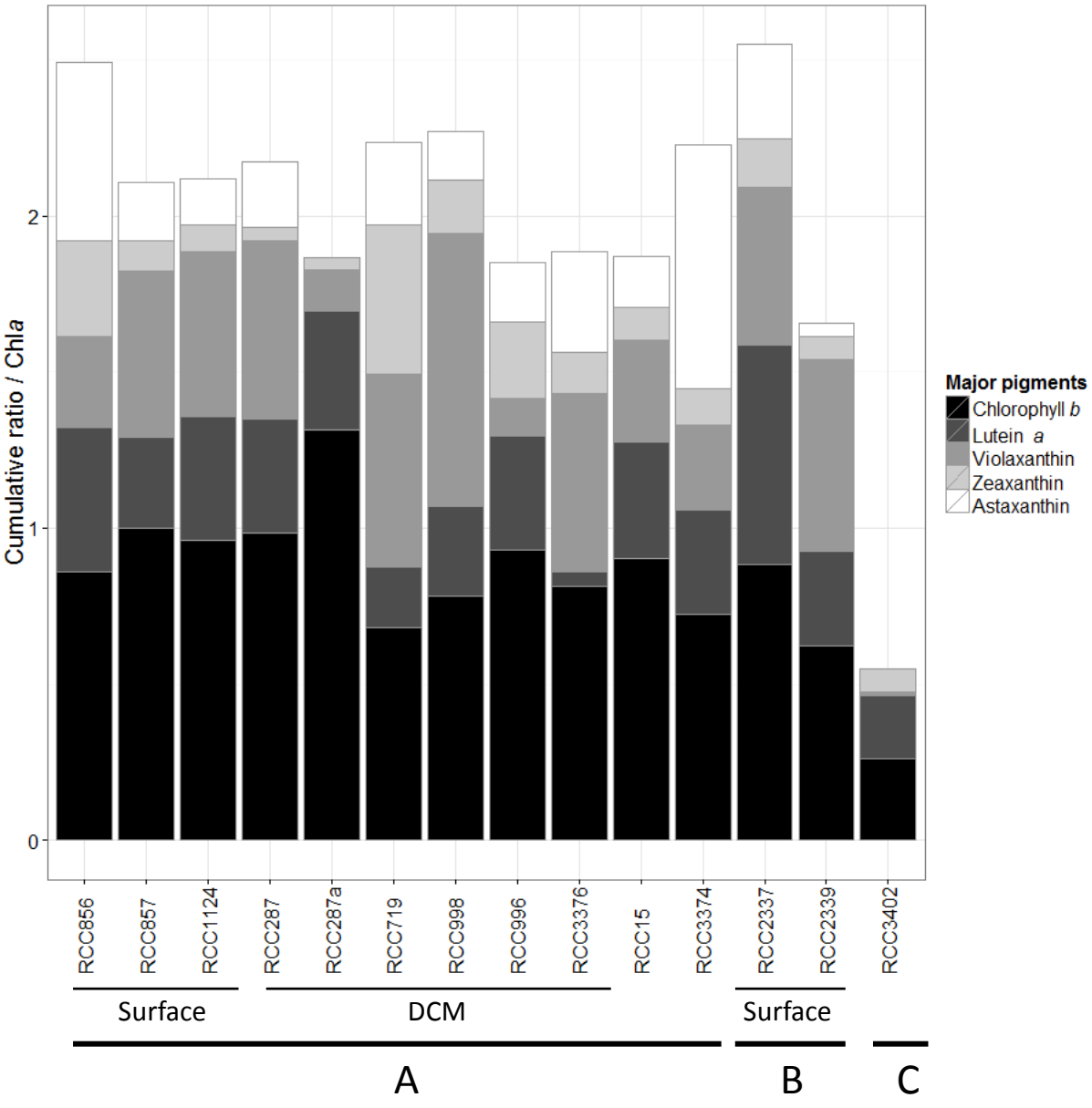
[a]Values reported by Latasa *et al.* 2004

Table S1

		Zapata et al. (2000)		Garrido et al. (2009)	
Pigment	Abbreviation	Rt (min)	Vis maxima (nm)	Rt (min)	Vis maxima (nm)
Chlorophyllide *b*	Chlide *b*	6.21	466, 647		
Chlorophyllide *a*	Chlide *a*	11.43	431, 665		
Loroxanthin	Lor	20.18	443, 468	4.81	448, 475
Neoxanthin	Neo	20.18	443, 468	5.07	438, 467
Violaxanthin	Viola	21.92	443, 472	5.39	442, 470
Astaxanthin	Asta	22.94	481	5.63	478
Antheraxanthin	Anth	25.76	447, 476		
Zeaxanthin	Zea	27.85	454, 481		
Lutein	Lut	28.03	447, 476		
Chlorophyll *b*	Chl *b*	32.04	463, 649		
Chlorophyll *a*	Chl *a*	33.6	432, 664		
βε -carotene	βε - car	35.82	449, 476		
ββ-carotene	ββ - car	36.23	454, 479		

Table S2

Strain	sub - clade	Light µE/m2/s	FCM Mean FL3-A /cell	fg Chl *a* / cell	Chl *b*	Chlide *a*	Chlide *b*	*Carotenoids*															
								Lor	*Lor %*	Neo	*Neo%*	Viola	*Viol%*	Asta	*Asta%*	Anth	*Anth%*	Zea	*Zea%*	Lut	*Lut%*	ββ - car	*ββ - car%*	βε - car	*βε - car%*
		140	97 734	4.99	0.986	0.106	0.000	0.024	*1.6*	0.167	*10.9*	0.572	*37.3*	0.210	*13.7*	0.024	*1.6*	0.043	*2.8*	0.363	*23.7*	0.051	*3.3*	0.079	*5.2*
RCC287	A	65	134 088	36.28	1.148	0.077	0.000	0.044	*2.9*	0.138	*9.1*	0.512	*33.9*	0.243	*16.1*	0.024	*1.6*	0.059	*3.9*	0.389	*25.8*	0.062	*4.1*	0.039	*2.6*
		14	187 046	20.13	1.041	0.000	0.000	0.071	*7.4*	0.100	*10.5*	0.282	*29.5*	0.085	*8.9*	0.019	*2.0*	0.032	*3.3*	0.230	*24.1*	0.089	*9.3*	0.047	*4.9*
		140	90 702	14.89	0.683	0.000	0.000	0.035	*1.8*	0.086	*4.4*	0.617	32.0	0.264	*13.7*	0.000	*0.0*	0.478	24.8	0.193	*10.0*	0.107	*5.5*	0.149	*7.7*
RCC719	A	65	101 762	22.74	0.832	0.000	0.000	0.066	*3.9*	0.118	*6.9*	0.722	42.7	0.137	*8.1*	0.044	*2.6*	0.194	11.5	0.204	*12.1*	0.140	*8.3*	0.069	*4.1*
		14	214 898	26.24	0.766	0.000	0.000	0.096	*7.6*	0.084	*6.6*	0.663	52.1	0.069	*5.4*	0.020	*1.6*	0.091	7.1	0.061	*4.8*	0.139	*10.9*	0.049	*3.9*
		140	145 875	23.37	0.860	0.000	0.000	0.113	*5.4*	0.079	*3.8*	0.291	14.0	0.570	*27.4*	0.042	*2.0*	0.310	14.9	0.461	*22.2*	0.075	*3.6*	0.136	*6.5*
RCC856	A	65	83 423	58.62	0.882	0.000	0.000	0.018	*0.9*	0.111	*5.6*	0.461	23.2	0.459	*23.1*	0.049	*2.5*	0.248	12.5	0.438	*22.1*	0.086	*4.3*	0.117	*5.9*
		14	231 009	56.88	0.942	0.000	0.000	0.096	*8.2*	0.102	*8.8*	0.310	26.6	0.138	*11.8*	0.023	*1.9*	0.093	8.0	0.222	*19.0*	0.133	*11.4*	0.049	*4.2*
		140	104 853	4.10	1.000	0.000	0.000	0.026	*1.9*	0.122	*8.7*	0.534	*38.2*	0.187	*13.4*	0.024	*1.7*	0.094	*6.7*	0.291	*20.8*	0.056	*4.0*	0.064	*4.5*
RCC857	A	65	150 099	11.46	1.128	0.000	0.000	0.072	*5.7*	0.131	*10.4*	0.400	*31.9*	0.141	*11.3*	0.025	*2.0*	0.072	*5.7*	0.314	*25.1*	0.065	*5.2*	0.034	*2.7*
		14	179 776	11.90	1.120	0.000	0.000	0.145	*13.6*	0.130	*12.2*	0.287	*27.0*	0.070	*6.6*	0.029	*2.7*	0.082	*7.7*	0.191	*18.0*	0.092	*8.7*	0.036	*3.4*
		140	253 221	51.57	0.931	0.074	0.000	0.043	*3.4*	0.095	*7.5*	0.119	9.5	0.191	*15.2*	0.039	*3.1*	0.245	19.5	0.364	*28.9*	0.091	*7.2*	0.074	*5.9*
RCC996	A	65	321 893	120.69	1.028	0.000	0.000	0.116	*9.8*	0.106	*9.0*	0.056	4.7	0.163	*13.8*	0.026	*2.2*	0.224	19.0	0.315	*26.7*	0.117	*9.9*	0.057	*4.8*
		14	418 516	61.80	0.968	0.000	0.000	0.195	*17.8*	0.120	*11.0*	0.399	36.5	0.049	*4.4*	0.016	*1.5*	0.058	5.3	0.077	*7.1*	0.179	*16.4*	0.000	*0.0*
		140	53 612	4.14	0.726	0.229	0.000	0.012	*0.7*	0.082	*4.5*	0.272	*14.8*	0.778	*42.3*	0.041	*2.3*	0.118	*6.4*	0.332	*18.1*	0.059	*3.2*	0.146	*7.9*
RCC3374	A	65	105 492	9.87	0.890	0.110	0.000	0.063	*4.8*	0.094	*7.1*	0.192	*14.5*	0.405	*30.5*	0.038	*2.9*	0.085	*6.4*	0.337	*25.4*	0.058	*4.3*	0.055	*4.1*
		14	136 153	11.33	0.777	0.000	0.000	0.113	*12.1*	0.098	*10.5*	0.176	*18.8*	0.187	*20.1*	0.020	*2.1*	0.035	*3.8*	0.159	*17.0*	0.082	*8.8*	0.063	*6.8*
		140	50 333	4.08	0.813	0.105	0.000	0.008	0.4	0.112	*5.7*	0.572	29.1	0.324	*16.5*	0.054	*2.7*	0.129	6.6	0.486	*24.7*	0.085	*4.3*	0.194	*9.9*
RCC3376	A	65	74 109	2.70	0.830	0.142	0.000	0.006	0.3	0.126	*6.7*	0.447	23.9	0.261	*14.0*	0.073	*3.9*	0.177	9.5	0.505	*27.1*	0.093	*5.0*	0.181	*9.7*
		14	101 238	10.08	0.783	0.053	0.000	0.005	0.5	0.095	*9.5*	0.108	10.8	0.091	*9.1*	0.029	*2.9*	0.130	13.0	0.365	*36.6*	0.055	*5.5*	0.122	*12.2*
		140	84 624	4.37	0.882	0.394	0.236	0.031	*1.4*	0.137	*6.3*	0.504	*23.1*	0.302	*13.8*	0.051	*2.3*	0.154	*7.0*	0.706	*32.3*	0.061	*2.8*	0.240	*11.0*
RCC2337	B	65	76 671	64.32	1.043	0.250	0.172	0.064	*4.2*	0.129	*8.4*	0.233	*15.3*	0.245	*16.1*	0.020	*1.3*	0.072	*4.7*	0.560	*36.7*	0.067	*4.4*	0.136	*8.9*
		14	213 378	18.09	1.040	0.158	0.118	0.109	*9.1*	0.137	*11.4*	0.255	*21.3*	0.058	*4.8*	0.028	*2.4*	0.067	*5.6*	0.332	*27.7*	0.118	*9.8*	0.095	*7.9*
		140	123 995	14.58	0.624	0.000	0.000	0.026	*1.7*	0.078	*5.1*	0.613	*40.4*	0.045	*3.0*	0.024	*1.6*	0.074	*4.8*	0.302	*19.9*	0.028	*1.8*	0.330	*21.7*
RCC2339	B	65	178 752	37.66	0.726	0.040	0.000	0.051	*3.6*	0.100	*7.1*	0.524	*37.4*	0.000	*0.0*	0.032	*2.3*	0.102	*7.3*	0.287	*20.5*	0.004	*0.3*	0.303	*21.6*
		14	223 724	75.86	0.736	0.000	0.000	0.051	*5.8*	0.076	*8.6*	0.264	*29.8*	0.031	*3.5*	0.025	*2.9*	0.051	*5.8*	0.193	*21.8*	0.033	*3.7*	0.160	*18.1*


Cumulative ratio / Chla
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1
2
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Violaxanthin
Zeaxanthin
Astaxanthin
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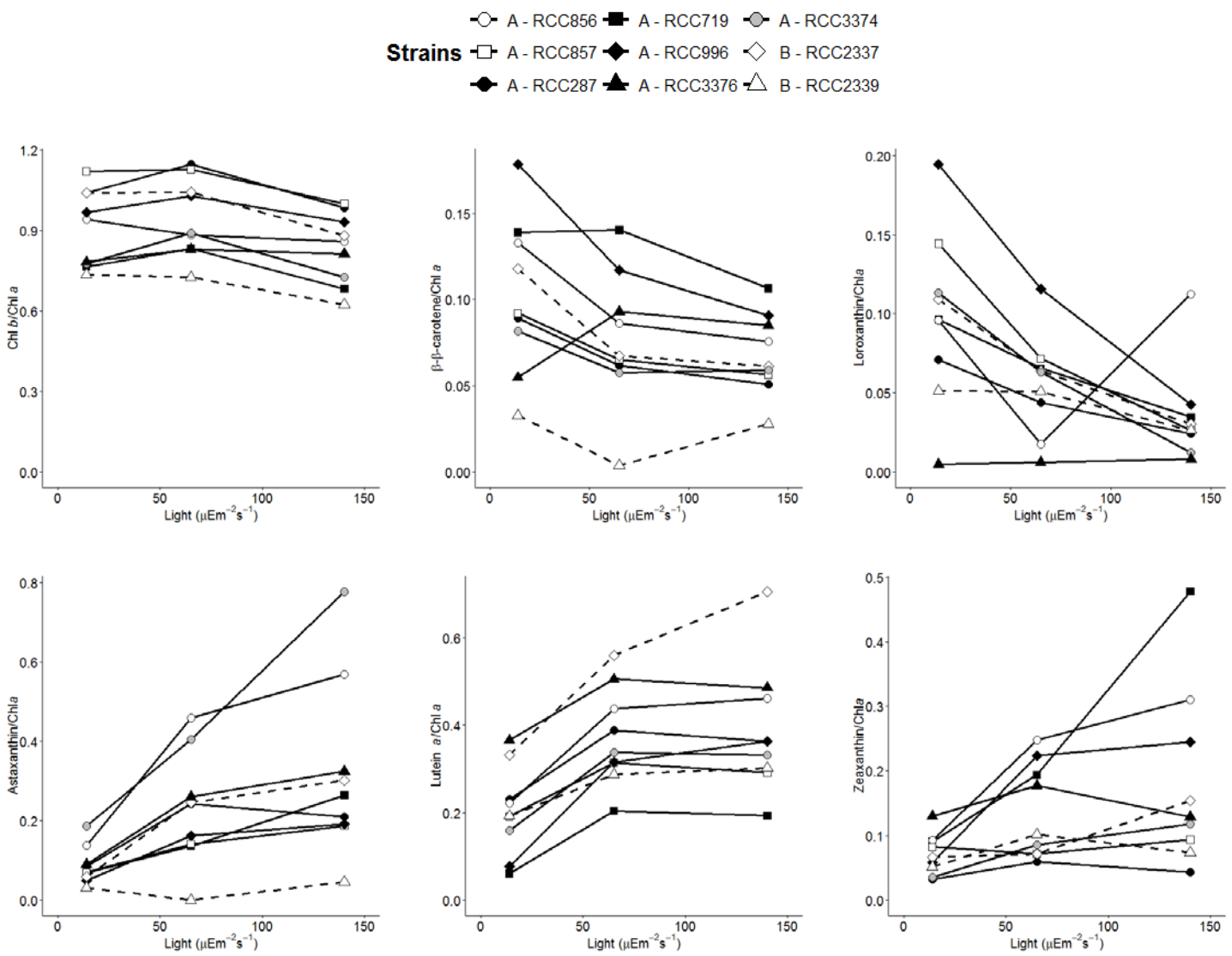
RCC857
RCC1124
RCC287
RCC287a
RCC719
RCC998
RCC996
RCC3376
RCC15
RCC3374
RCC2337
RCC2339
RCC3402
Surface
DCM
Surface
A
B
C

Figure 1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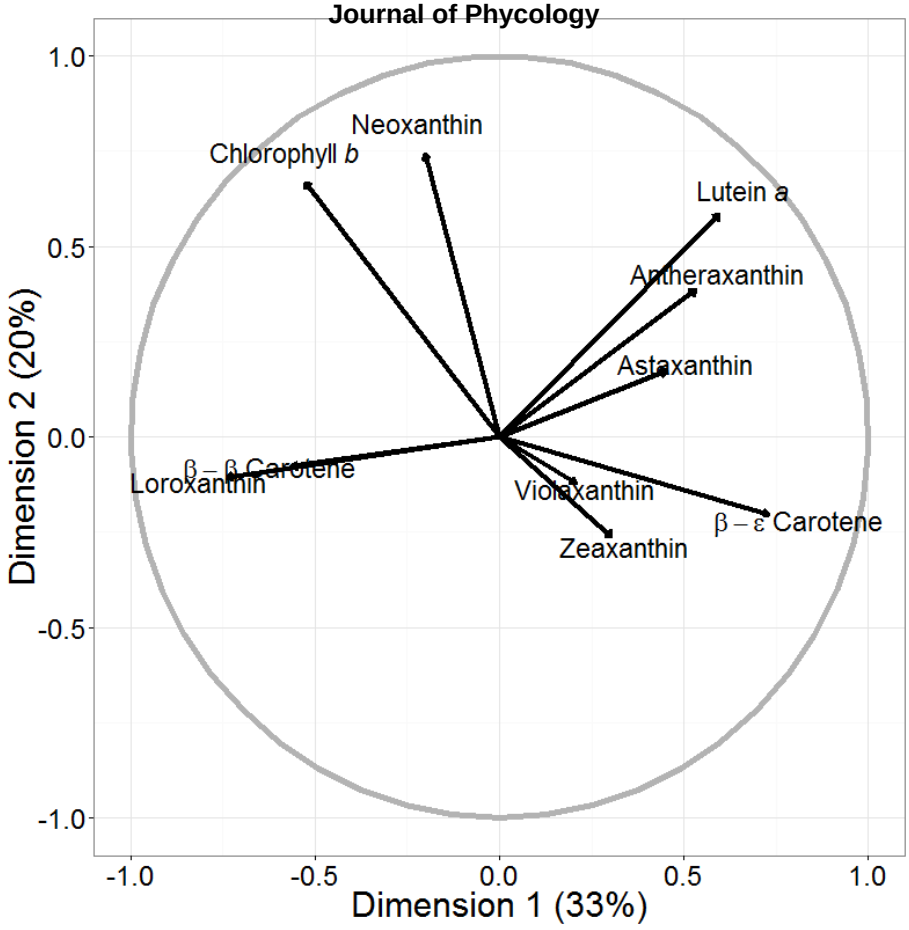
A - RCC3374
A - RCC857
A - RCC996
B - RCC2337
A - RCC287
A - RCC3376
B - RCC2339
Chl b/Chl a
β-β-carotene/Chl a
Loroxanthin/Chla
Astaxanthin/Chla
Lutein a/Chl a
Zeaxanthin/Chla
Light (μEm⁻²s⁻¹)

Figure 2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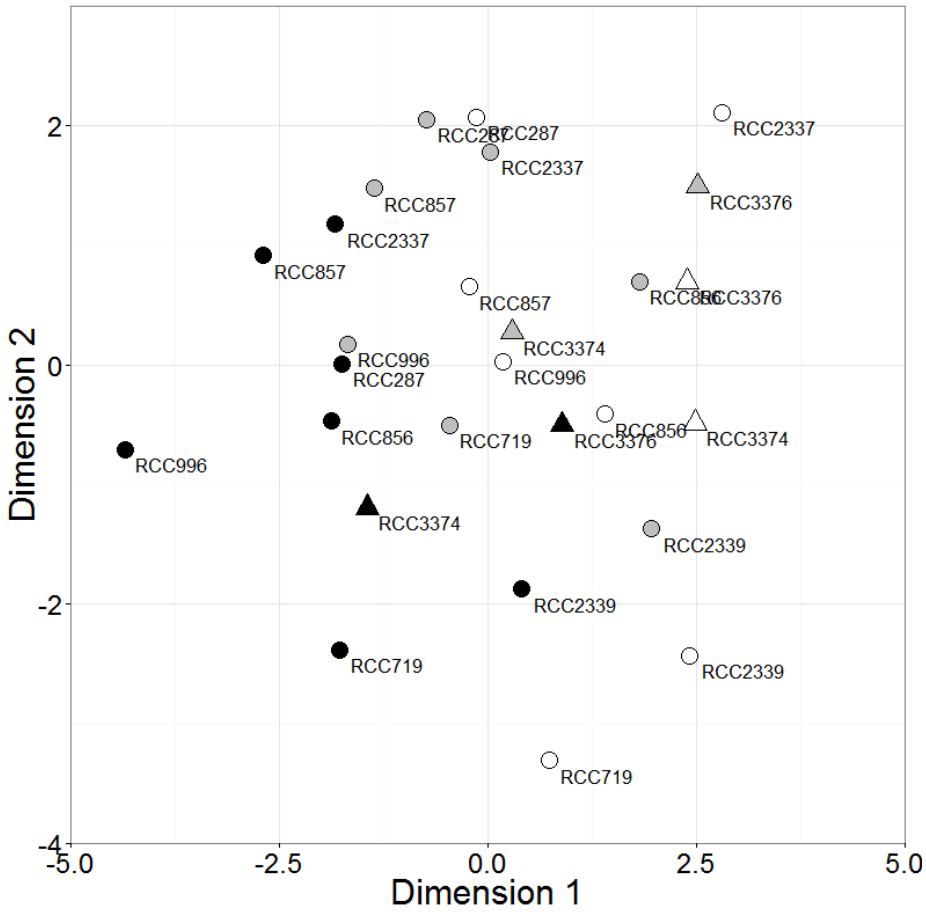
Chlorophyll b
Lutein a
Antheraxanthin
Astaxanthin
β – β Carotene
Loroxanthin
Violaxanthin
β – ε Carotene
Zeaxanthin
Dimension 2 (20%)
Dimension 1 (33%)
1.0
0.5
0.0
-0.5
-1.0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RCC3376
RCC3374
RCC996
RCC3374
RCC2339
RCC2339
RCC719
RCC2339
RCC719
Dimension 2
Dimension 1
2
0
-2
-4
-5.0
-2.5
0.0
2.5
5.0

Figure 3

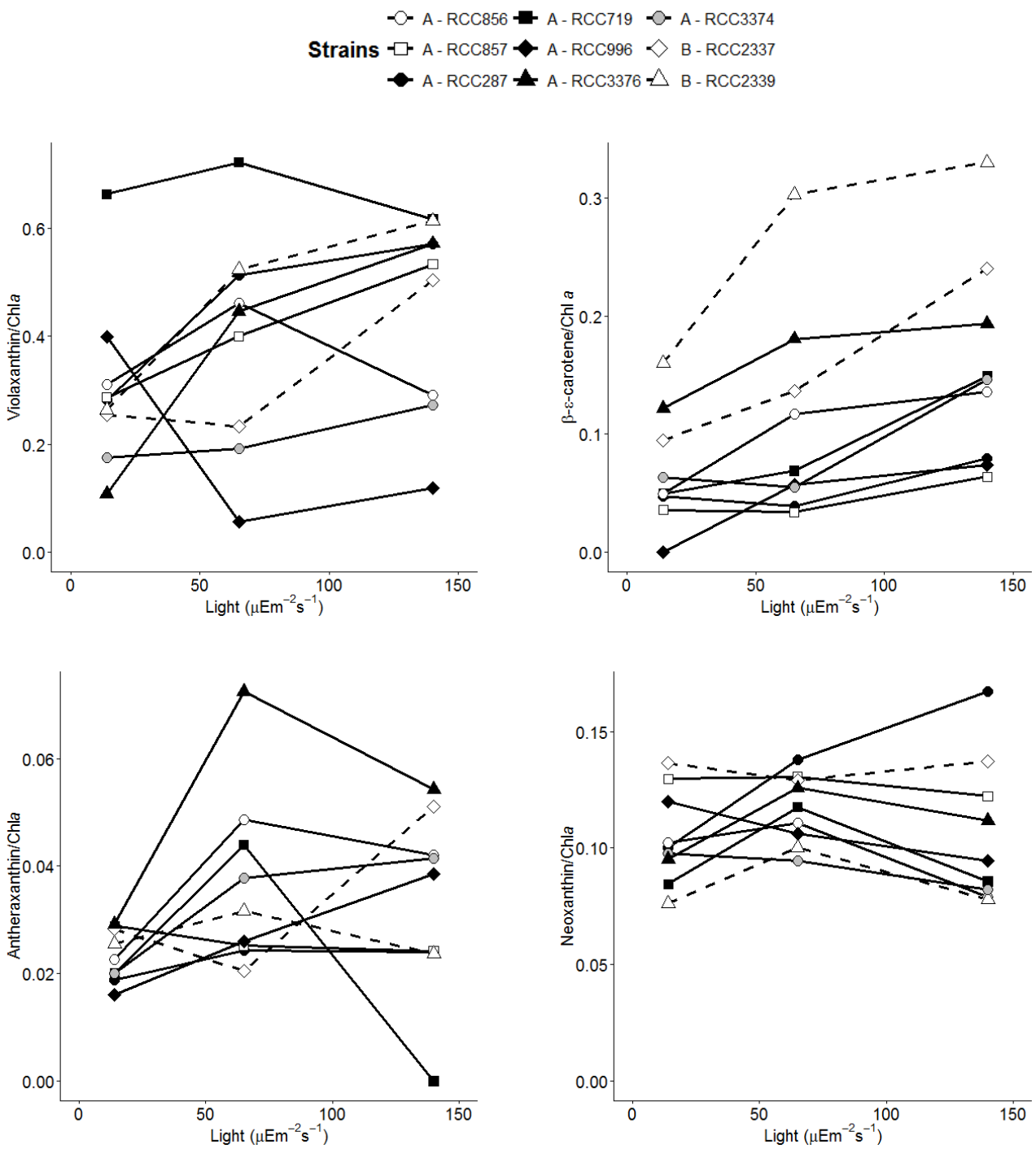
Strains
A - RCC856
A - RCC719
A - RCC3374
A - RCC857
A - RCC996
B - RCC2337
A - RCC287
A - RCC3376
B - RCC2339
Violaxanthin/Chla
β-ε-carotene/Chl a
Antheraxanthin/Chla
Neoxanthin/Chla
Light (μEm⁻²s⁻¹)

Figure S1.